DEPARTMENT OF THE AIR FORCE
59TH MEDICAL WING (AETC)
JOINT BASE SAN ANTONIO - LACKLAND TEXAS

6 FEB 2017

MEMORANDUM FOR SGOZ
ATTN: MAJ BRYANT J. WEBBER

FROM: 59 MDW/SGVU

SUBJECT: Professional Presentation Approval

1. Your paper, entitled **Prevalence and Seroprevalence of Trypanosoma Cruzi Infection in A Military Population in Texas** presented at/published to **AM J Trop Med Hyg (if rejected, then Emerging Infectious Dis, J Infect Dis, PLoS Neglected Trop Dis, or Am J Prev Med)** in accordance with MDWI 41-108, has been approved and assigned local file #**17056.**

2. Pertinent biographic information (name of author(s), title, etc.) has been entered into our computer file. Please advise us (by phone or mail) that your presentation was given. At that time, we will need the date (month, day and year) along with the location of your presentation. It is important to update this information so that we can provide quality support for you, your department, and the Medical Center commander. This information is used to document the scholarly activities of our professional staff and students, which is an essential component of Wilford Hall Ambulatory Surgical Center (WHASC) internship and residency programs.

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Linda Steel-Goodwin

LINDA STEEL-GOODWIN, Col, USAF, BSC
Director, Clinical Investigations & Research Support

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	Bryant J. Webber, Maj, USAF, MC, 559 THLS/SGOZ	☐ YES ☒ NO	FWH20140074H

5. PROTOCOL TITLE: (**NOTE:** For each new release of medical research or technical information as a publication/presentation, a new 59 MDW Form 3039 must be submitted for review and approval.)

The Impact of Chagas Disease on Military Training in Texas

6. TITLE OF MATERIAL TO BE PUBLISHED OR PRESENTED:

Prevalence and Seroprevalence of Trypanosoma cruzi Infection in a Military Population in Texas

7. FUNDING RECEIVED FOR THIS STUDY? ☒ YES ☐ NO FUNDING SOURCE: AFMSA

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14. 59 MDW PRIMARY POINT OF CONTACT (Last Name, First Name, M.I., email)	15. DUTY PHONE/PAGER NUMBER
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18. AUTHOR'S PRINTED NAME, RANK, GRADE	19. AUTHOR'S SIGNATURE	20. DATE
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26. DATE REVIEWED January 31, 2017		27. DATE FORWARDED TO 502 ISG/JAC

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IRB approved study with all appropriate disclaimers, and no COI noted. Approved

30. PRINTED NAME, RANK/GRADE, TITLE OF REVIEWER	31. REVIEWER SIGNATURE	32. DATE
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48. PRINTED NAME, RANK/GRADE, TITLE OF REVIEWER	49. REVIEWER SIGNATURE	50. DATE

Prevalence and Seroprevalence of *Trypanosoma cruzi* Infection in a Military Population in Texas

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Abstract

Hundreds of triatomine insects were recently collected at field training sites on Joint Base San Antonio, a large military installation located in south-central Texas. Over 26% of these triatomines tested positive for *Trypanosoma cruzi*, the causative parasitic agent of Chagas disease, and 33% had detectable human blood in their midgut. Given the potential for vector-borne human Chagas disease, a cross-sectional study was conducted to determine the prevalence and seroprevalence of *T. cruzi* infection in highest risk subpopulations on the installation, including students and instructors who work and sleep in triatomine-endemic field settings. Real-time polymerase chain reaction, enzyme-linked immunosorbent assay, and indirect immunofluorescent assay were performed on enrolled subjects (N=1,033), none of whom tested positive for *T. cruzi* or anti-*T. cruzi* antibodies. Current countermeasures employed during field training on Joint Base San Antonio appear to be sufficient for preventing autochthonous human Chagas disease.

Introduction

Chagas disease, or American trypanosomiasis, is caused by infection with *Trypanosoma cruzi*. The protozoan parasite is transmitted to humans most commonly through the infected excreta of hematophagous triatomine insects of the family Reduviidae, entering the bloodstream through a wound or mucous membrane.[1] Known colloquially as "kissing bugs," triatomines are found throughout the Western hemisphere. Eleven species are endemic to the southern United States, most of which are competent vectors of the parasite.[2] In addition to the vector-borne route, *T. cruzi* may be transmitted congenitally, orally in contaminated food or beverages, or directly via blood transfusion or organ and tissue transplantation.[3]

The majority of human cases are subclinical in both the acute and chronic stages, resulting in lifelong, undiagnosed infection. Cardiac disease, gastrointestinal disease, or both develop in approximately one-third of cases, typically manifesting years or decades after initial infection.[4] Chagas disease mortality is usually attributed to heart failure or ventricular arrhythmia,[5] but even asymptomatic infection with *T. cruzi* may increase all-cause mortality risk.[6]

53 Between 5 and 8 million people globally are infected with *T. cruzi*,[1,7] incurring an annual
54 economic burden of 7.2 billion USD.[8] Although vectorial transmission is restricted to the Americas,[9]
55 human migration from endemic to non-endemic countries—and within endemic countries from rural to
56 urban areas—has broadened the distribution of prevalent Chagas disease.[10] Both imported and
57 autochthonous cases occur in the United States, with the former predominating: at least 240 thousand
58 Latin American immigrants are presumably infected,[11] whereas fewer than 30 locally-acquired infections
59 have ever been reported.[12] The dearth of documented autochthonous cases, however, may be more
60 indicative of provider unawareness and suboptimal surveillance than true disease incidence.[12,13]
61 Recognizing the potential for vector-borne transmission across a broad swath of the southern United
62 States, the Centers for Disease Control and Prevention (CDC) prioritizes Chagas as one of five neglected
63 parasitic infections[14] and urges more research to define autochthonous infection risk.[12]
64 In the greater San Antonio metropolitan area and throughout south-central Texas, Chagas disease
65 has been a known but vaguely defined human disease threat since at least the 1960s.[15-17] An obligation to
66 elucidate that threat recently emerged from several biosurveillance findings on Joint Base San Antonio
67 (JBSA), one of the largest military training installations in the United States. A 2007 serosurvey of
68 military working dogs, all of which are trained on JBSA, found that 24 (8%) harbored anti-*T. cruzi*
69 antibodies. Multiple military working dogs serving in Iraq required evacuation due to cardiomyopathy,
70 later attributed to Chagas disease.[18] A faunal survey was commissioned, which found that 43% (88/205)
71 of collected adult triatomines and 22% (163/736) of nymphs tested positive for *T. cruzi* on polymerase
72 chain reaction (PCR), and blood meal analysis revealed that 33% (43/131) contained human blood in their
73 midgut. Among adults, *Triatoma sanguisuga* (66%) and *Triatoma gerstaeckeri* (30%) were the most
74 common species identified (Daniels, C., unpublished data). This prompted the enforcement of new
75 administrative, technical, and personal protective measures—as well as the reinforcement of existent
76 measures—to protect humans and dogs against vector-borne pathogen exposure during field exercises on
77 JBSA. Since triatomines were often collected in close proximity to high volume training sites, including
78 within field training tents, we also initiated this study to determine human infection risk.

Materials and Methods

This cross-sectional study was designed to establish the prevalence of *T. cruzi* parasitemia and seroprevalence of anti-*T. cruzi* antibodies in five subpopulations most at risk for vector-borne infection while training and working on the installation: students graduating from the US Air Force Security Forces Apprentice course, all of whom had spent 3 weeks training outdoors in a triatomine-endemic area in the month prior to study enrollment, and most of whom had completed a week-long field training exercise on a separate triatomine-endemic site of the installation; instructors from the US Air Force Security Forces Apprentice course; instructors from the US Air Force Basic Military Training field training course; instructors from the Department of Defense Military Working Dog school; and instructors from the US Air Force Survival, Evasion, Resistance, and Escape course. Given reduced prevalence of triatomines during the winter months,[19] and thus reduced likelihood of detecting parasitemia and anti-*T. cruzi* IgM antibodies, we suspended enrollment from December through March.

We administered a questionnaire to all consented participants in order to gather demographic data, quantify exposure risk, and ascertain the geographic location of infection, should a subject test positive. Demographic data included age, sex, and self-reported race and ethnicity. The questionnaire initially focused on vectorial transmission risk by extracting information regarding military training; permanent residence in and travel to triatomine-endemic areas;[1,2] camping, hunting,[20] and exposure to reservoir wildlife[3,17] in triatomine-endemic areas; and bites by triatomines or by unidentified insects that may have been triatomines. We displayed high-resolution photos of *T. sanguisuga* and *T. gerstaeckeri* adults to facilitate an accurate bite history. After discussing preliminary results with two external consultants, we added questions pertaining to blood transfusion and congenital transmission routes.

We collected whole blood from consented volunteers by peripheral venipuncture. On all subjects from whom we could obtain sufficient aliquots, we performed real-time PCR to determine the prevalence of *T. cruzi* parasitemia and an enzyme-linked immunosorbent assay (ELISA) and indirect immunofluorescent antibody (IFA) test to determine the seroprevalence of anti-*T. cruzi* antibodies.

PCR was conducted per CDC published methodology, utilizing the same primers and probes.[21] Two multiplex TaqMan assays were performed in parallel targeting three highly conserved and repetitive *T. cruzi* genomic regions: nuclear mini-satellite TCZ; kinetoplast DNA; and the small subunit ribosomal RNA (18S rRNA) gene. An internal validation study was completed using human blood spiked with known amounts of *T. cruzi* epimastigotes. The test was considered positive if all three targets were positive.

The Chagatest ELISA recombinante v.3.0 (Wiener Laboratórios, Rosario, Argentina) was performed, as directed by the manufacturer, for detecting human IgG and IgM anti-*T. cruzi* antibodies. Collected serum samples were incubated with immobilized antigen, washed, incubated with goat anti-human IgG conjugated to horse radish peroxidase, and washed again. Tetramethylbenzidine and hydrogen peroxide were then added, and the reactions were stopped with 2 N sulfuric acid. The colorimetric readings were taken at 450 nm in a plate reader, using a reference wavelength of 650 nm. Cut-off values were defined, per manufacturer guidance, as the mean negative control readings plus 0.3. The test was considered positive if greater than the cut-off value plus 10%, negative if less than the cut-off value minus 10%, and equivocal if within ± 10% of the cut-off value.

Sera were also evaluated for the presence of human IgG antibodies against *T. cruzi* via IFA technique. A positive and negative control were validated from the 21-member panel of the SeraCare Life Sciences Chagas Titer AccuSet™. Panel member 1, which had an antibody titer of 1:4096, was used as a positive control at a 1:200 dilution. Panel member 21, which had a negative titer (<1:128), was used as a negative control, also at a 1:200 dilution. Subject samples were screened at a 1:128 dilution. Dilutions were placed on *T. cruzi* antigen-coated microscope slides, washed to remove unbound serum antibodies, stained with a fluorescein isothiocyanate-labeled goat anti-human IgG conjugate, and visualized through a fluorescence microscope. A sample was considered positive at a titer equal to or greater than 1:128

We used descriptive statistics to build demographic and exposure profiles of the enrolled sample, both collectively and stratified by student and instructor status. We compared exposure time between students and instructors with an unpaired t-test, using Epi Info v7.0 (CDC, Atlanta, GA). This study was

130 approved by the 59th Medical Wing Institutional Review Board (FWH20140074H). Written informed
131 consent was obtained from all subjects.

132 **Results**

133 A total of 1,033 subjects were enrolled. Consistent with the ratio of students to instructors on the
134 installation, the vast majority of subjects (93.1%) were students graduating from the Security Forces
135 Apprentice course (Table 1). During the 16-month study period (April-November 2015 and April-
136 November 2016), we enrolled approximately 15% and 30% of eligible students and instructors,
137 respectively. Most subjects were male (76.9%) and white, non-Hispanic (54.8%). The mean (standard
138 deviation [SD]) age was 21.6 (4.6) years. Three subjects experienced presyncope with venipuncture, all of
139 whom recovered fully without medical intervention. No other adverse events were noted.

140 Five subjects (0.5%) reported a triatomine bite and 131 (12.7%) reported a bite from an
141 unidentified insect that may have been a triatomine. Subjects experienced 8,130 weeks of total exposure
142 time in the triatomine-endemic field environment of JBSA, for a mean (SD) of 7.7 (18.0) weeks.
143 Instructors (47.0 [45.6] weeks) had a greater mean exposure time than students (4.0 [0.4] weeks)
144 ($p<0.001$). Details on demography, binary risk factors, and time residing and conducting higher risk
145 activities in triatomine-endemic areas are provided in Table 1.

146 All PCR (n=1017), ELISA (n=1023), and IFA (n=1023) tests were negative, with the exception
147 of one equivocal ELISA result. The enrollment total exceeds laboratory result figures because adequate
148 blood specimens could not be obtained on every subject. The indeterminate ELISA result (0.279 IV
149 [equivocal range: 0.27-0.33 IV]) belonged to a student of Hispanic ethnicity, who was born and lived in
150 Central America for 2 years before emigration to the United States. He then lived in the southwest United
151 States for 19 years prior to arrival at JBSA. During training he experienced three bites that may have been
152 from triatomines, although he could not definitively classify the insect. Repeat ELISA testing was also
153 equivocal, and his PCR and IFA testing were negative. He was advised to visit his health care provider for
154 further discussion and workup.

155 **Discussion**

Of 1,033 enrolled subjects, none tested positive for either *T. cruzi* parasites or anti-*T. cruzi* antibodies, suggesting that Chagas disease is currently a negligible threat for military personnel on JBSA. One subject, who tested negative on PCR and IFA, had an equivocal ELISA result. Even if he were truly infected, his case could not be conclusively categorized either as autochthonous, because of his early childhood spent in Latin America, or as vectorial, given the possibility of vertical transmission.

The apparent mismatch between these reassuring findings and the troubling biosurveillance signals—to include cases of canine Chagasic cardiomyopathy and a high volume of *T. cruzi*-infected triatomines—could have several explanations. Exposure of our study subjects to triatomines may have been limited by several anteceding countermeasures: vegetation reduction[22] and application of pyrethroid-based insecticides[2] around tents and field training sites; aggressive reduction of zoonotic reservoirs, particularly woodrats[23] and feral swine;[24] requirements pertaining to field uniform wear (i.e., long pants bloused under boots and long sleeves secured at the wrist with buttons); distribution of DEET-based insect repellents free of charge; and, specifically for students during their field training exercise, sleeping in permethrin-treated bed nets.[25]

These countermeasures alone cannot explain our negative findings, however, since one-third of triatomines collected on our field training sites had fed on humans, and over 13% of our subjects reported bites by triatomines or unidentified insects. Other protective factors may have contributed. First, the stercorarian transmission of parasite from vector to human host is inefficient. Statistical modeling estimates that one case of vectorial transmission requires 900-4,000 contacts with an infected triatomine.[26] Second, triatomines opportunistically feed on a variety of vertebrate species—many of which are present on JBSA and have tested positive for *T. cruzi* infection in south-central Texas[27,28]—offering competitive, non-human blood meal sources. Third, vector competency for any given triatomine species depends on several factors, including environmental distribution, flying and dispersal capacity, inclination to invade human dwellings, and feeding-to-defecation interval.[2,29,30] Unlike *Triatoma infestans*, *Triatoma dimidiata*, and *Rhodnius prolixus*, the predominant *T. cruzi* vectors in South America,[2,3] the sylvatic species indigenous to the southern United States are less likely to defecate while taking a blood meal[31,32] and to

182 colonize domestic and peridomestic settings.[2] These biologic and ecologic dynamics may partially
183 explain why autochthonous human Chagas disease appears to be uncommon in the United States,[12]
184 despite a high prevalence of *T. cruzi* infection in triatomine vectors[18,19,33-35] and substantial evidence for
185 triatomine feeding on humans.[33,36,37] This incongruity between a high prevalence of triatomines and low
186 seroprevalence of human *T. cruzi* infection was also reported on the Yucatan Peninsula of Mexico.[38]
187 Another explanation, albeit a study limitation and not a causative factor, is the possibility of false
188 negative testing. According to a recent meta-analysis, the Weiner ELISA has a sensitivity of 93.7% (95%
189 confidence interval: 87.7%, 96.9%) for detecting human antibodies against *T. cruzi*.[39] IFA testing is
190 approximately 90% sensitive, although substantial heterogeneity exists across studies.[40] This may be due
191 to methodological differences, particularly with titer dilution cutoffs used to determine positive results.
192 Our 1:128 dilution screening was designed to maximize overall test accuracy, but other laboratories may
193 demarcate a positive result at a titer of 1:80, [41] thus conceding some specificity for improved sensitivity.
194 PCR, which is most valuable diagnostically during the parasitemic acute stage of disease,[4] has a
195 sensitivity below 50% for detecting chronic infection in adults.[39] However, by utilizing 3 tests in parallel
196 and analyzing over a thousand subjects, imperfect sensitivity for any one test does not undermine
197 conclusions drawn this study.
198 The lack of molecular and serologic evidence of *T. cruzi* infection in our study sample, though
199 encouraging, should not be misapplied. First, our findings should not be used to exclude Chagas disease
200 from the differential diagnosis list when evaluating service members who trained or worked on
201 triatomine-endemic field sites of JBSA. Although we selected populations with the highest risk of
202 exposure, we only tested a sample thereof, and our results may not be generalizable to populations on the
203 installation before aggressive countermeasures were deployed. Second, our findings should not be used to
204 establish countermeasure success. In the absence of a control group not employing these measures, our
205 study was not designed to verify their effectiveness. Current countermeasures appear sufficient but not
206 categorically necessary in preventing autochthonous human Chagas disease on JBSA.

Despite an abundance of *T. cruzi*-infected triatomine vectors, some of which evidently feed on humans, Chagas disease is currently not a major infectious disease threat for military students and instructors on JBSA. Even if parasite transmissibility is intrinsically unlikely due to bioecological factors, primary preventive measures reducing exposure to *T. cruzi* and other vector-borne pathogens should continue. In order to stage realistic military field training exercises while maximizing the health of humans, animals, and the environment, we urge holistic One Health approaches built on collaboration between military training leadership, civil engineers, and medical, veterinary, and public health personnel.

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383 **Table 1. Demographic and risk factor profile of study subjects (N=1,033)**

	Students N=962	Instructors N=71	Total N=1,033
Population			
-Security Forces Apprentice course students	962 (100%)		962 (93.1%)
-Security Forces Apprentice course instructors		2 (2.8%)	2 (0.2%)
-Basic Military Training field training instructors		36 (50.7%)	36 (3.5%)
-Military Working Dog School instructors		23 (32.4%)	23 (2.2%)
-Survival, Evasion, Resistance, and Escape instructors		10 (14.1%)	10 (1.0%)
Age, mean (std dev)	20.9 (3.6)	31.6 (5.0)	21.6 (4.6)
Sex			
-Male	735 (76.4%)	59 (83.1%)	794 (76.9%)
-Female	227 (23.6%)	12 (16.9%)	239 (23.1%)
Race/ethnicity			
-White, non-Hispanic	515 (53.5%)	51 (71.8%)	566 (54.8%)
-Black, non-Hispanic	121 (12.6%)	7 (9.9%)	128 (12.4%)
-Hispanic	221 (23.0%)	8 (11.3%)	229 (22.2%)
-Other	105 (10.9%)	5 (7.0%)	110 (10.6%)
Potential exposures			
-Known triatomine bite	4 (0.4%)	1 (1.4%)	5 (0.5%)
-Unidentified insect bite	102 (10.6%)	29 (40.8%)	131 (12.7%)
-Received blood products in US*	7 (0.8%)	3 (5.9%)	10 (1.1%)
-Received blood products outside US*	3 (0.3%)	0	3 (0.3%)
-Mother lived in Latin America before birth*	90 (10.2%)	4 (7.8%)	94 (10.1%)
Weeks in triatomine-endemic area, mean (std dev)			
-Field environment at JBSA-Lackland	4.0 (0.4)	47.0 (45.6)	7.7 (18.0)
-Camping/hunting in Latin America or southwest US†	30.8 (125.0)	75.8 (283.5)	35.2 (147.0)
-Wildlife exposure‡ in Latin America or southwest US†	110.2 (280.9)	246.1 (513.7)	126.7 (320.2)
-Living/traveling in Latin America	78.0 (230.1)	80.9 (225.2)	67.3 (211.6)
-Living/traveling in southwest US†	430.4 (485.5)	238.9 (379.4)	380.9 (474.4)

384
385 *N=931 since these questions were added to the questionnaire after study initiation.
386
387 †Southwest United States was defined as Arizona, California, Colorado, Nevada, New Mexico,
388 Oklahoma, Texas, and Utah.
389
390 ‡Wildlife exposure was defined as either hunting or living in a dwelling infested by woodrats, raccoons,
391 opossums, skunks, wild hogs, coyotes, or deer.